Gas Chromatography-Mass Spectrometry Analysis of Polycyclic Aromatic Hydrocarbons in Single Puff of Cigarette Smoke

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Abstract

A single puff polycyclic aromatic hydrocarbon (PAH) analysis technique was developed in our laboratory and applied to study PAH formation in each puff during the cigarette smoking process. An impaction trap was used to collect the total particulate matter (TPM) from a single puff of smoke. The TPM was then weighed and dissolved in a 5:5:1 mixture of toluene, hexane, and isopropanol. Five PAHs, including naphthalene, phenanthrene, fluoranthene, pyrene, and benzo[a]pyrene were analyzed by gas chromatography-mass spectrometry using selective ion monitoring mode. This technique offers the capability to analyze trace amounts of PAHs in a single puff of mainstream cigarette smoke and provides information on PAH formation from each puff. Comparison of relative PAH levels in the lighting puff of mainstream cigarette smoke using different types of lighters is presented to illustrate the importance of applying the single puff analysis technique in understanding smoke chemistry.

Keywords: PAHs; benzo[a]pyrene; tobacco; mainstream smoke; cigarettes

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Introduction

Polycyclic aromatic hydrocarbons (PAHs) have been observed by numerous workers in tobacco smoke [1-7]. Over 300 of these compounds, many of them methylated, have been identified. The PAHs are generally found in trace amounts in the particulate matter of smoke in the presence of a very complex matrix. The analysis of PAHs in cigarette smoke usually involves solvent extraction from total particulate matter, solid phase extraction, cleaning, and re-concentration before analysis. Chromatography and spectrometry techniques are commonly used for the analysis. Because of the extensive extraction and cleaning steps involved, several cigarettes need to be smoked to collect enough total particulate matter (TPM) for the mainstream PAH analysis. PAH levels in mainstream smoke are typically reported as an average for one cigarette. To our knowledge, information on PAH levels in each individual puff of a cigarette has not been reported in the literature except for one recent publication from this laboratory [8].

In the present paper, we describe a technique that allows polycyclic aromatic hydrocarbon analysis from a single puff of mainstream cigarette smoke. This technique was first developed in our laboratory to study PAH formation and distribution in each puff during the cigarette smoking process. It offers the capability to analyze trace amounts of PAHs in a single puff of cigarette smoke and provides information on PAH formation from each puff. Exploring the smoking process on a single puff basis has recently been reported and shown to be essential in terms of providing insight into the smoke chemistry and increasing our understanding of combustion and pyrolysis in cigarette smoking [8-10]. Since some compounds in tobacco smoke vary from one puff to another, only a single puff analysis technique can reveal the changes happening in each puff. For example, the tobacco rod length, the amount of smoke condensate deposited on cigarette tobacco rod and filter, and whether or not the tobacco has been exposed to the previous puff were found to influence single puff mainstream smoke formaldehyde levels [9]. To illustrate the importance of applying single puff analysis technique in smoke research, we present here a comparison of relative PAH levels in the lighting puff of mainstream cigarette smoke using different types of lighters.

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Experimental

Instrumentation The single puff mainstream PAH analysis was performed on a Hewlett-Packard 6890 gas chromatograph equipped with a 5973 mass spectrometry (MS) detector. The GC column was a J&W DB-17ms (J&W, 30 meter x 0.25 mm ID x 0.25 µm film thickness). The MS detector was set in selected ion monitoring (SIM) mode and electron impact ionization (electron energy 70 eV) was used to generate the ions. The oven temperature program was as follows: initial temperature at 50 °C for 1 minute, to 195 °C at 10 °C/min, to 280 °C at 5 °C/min, hold 10 minutes, and to 310 °C at 30 °C/min, hold 15 minutes. The injector temperature was set at 320 °C and the flow rate at 1.0 ml/minute. MS quadrupole temperature was set at 106 °C, MS source temperature at 230 °C, and transfer line temperature at 300 °C.

Smoke sampling All smoking, unless otherwise noted, was done using a single-port smoking machine (KC Automation, Richmond, VA) with a square wave profile, 35-ml volume, 2-second duration, and 60-second interval between puffs. Mainstream smoke is defined as the smoke exiting the mouthend of the cigarette and immediately drawn into a collection device [11]. The cigarettes used in this study were University of Kentucky Research Cigarette 1R4F, developed for research purposes by the National Cancer Institute of the National Institute of Health, the Agricultural Research Service of the United States Department of Agriculture, and the Tobacco and Health Research Institute of the University of Kentucky [12], IM16, an industrial monitor cigarette designed by Philip Morris, as well as two other non-commercial research cigarettes, 100% burley tobacco 99.KF.146 and 100% bright tobacco 99.KF.182, also developed for research purposes by Philip Morris. The cigarettes, unless otherwise noted, were lit with a Borgwaldt electric lighter using the medium setting. For the lighting puff (*i.e.* the first puff), the end of the cigarette rod was kept in contact with the heat source during the entire puffing period (2 seconds). For each puff, the total particulate matter was collected on a clean small aluminum foil disc (about 6 mm in diameter) placed inside a collecting vial, 8 mm i.d. and 35 mm long, in an impaction trap. The disc was then

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removed from the collecting vial and weighed. The weight difference of the aluminum disc before and after the puff determines the quantity of total particulate matter produced. The TPM deposited was then dissolved in 50 μl of a 5:5:1 mixture of toluene, hexane, and isopropanol containing 1 μg/ml of each of four deuteraded PAHs, naphthalene-d8, phenanthrene-d10, chrysene-d12 and perylene-d12, as internal standards. The TPM/solvent mixture was sonicated at room temperature for 10 minutes and then 1 μl of the solution was injected into the GC-MS in a splitless mode with no further dilution. The small solvent volume used allows detection of the small quantities of PAH present in a single puff. Furthermore, since no further sample treatment was performed in this method, it eliminated the sample loss associated with extraction and cleaning, and greatly reduced the analysis time. The main disadvantage of a reduced sample treatment is that it requires more frequent instrument maintenance since the mass spectrometer ion source and the GC column get contaminated faster.

PAH analysis Five PAHs were analyzed in this study: naphthalene, phenanthrene, fluoranthene, pyrene, and benzo[a]pyrene. For each PAH compound, three characteristic ions were monitored in the selected ion monitoring (SIM) mode (naphthalene, $C_{10}H_8$: *m/z* 128, 102, 64; phenanthrene, $C_{14}H_{10}$: *m/z* 178, 152, 76; pyrene and fluoranthene, $C_{16}H_{10}$: *m/z* 202, 101, 88; and benzo[a]pyrene, $C_{20}H_{12}$: *m/z* 252, 126, 113). Since the ratio of mass fragments to parent ion is characteristic for each compound, using this approach allowed us to assign the retention time for each PAH under investigation unequivocally. This is particularly important for the isomers fluoranthene and pyrene. After this assignment, only the parent ions were used in the subsequent data analysis. Only the parent ions of the internal standards were monitored (naphthalene-d8, $C_{10}D_8$: *m/z* 136; phenanthrene-d10, $C_{14}D_{10}$: *m/z* 188; chrysene-d12, $C_{18}D_{12}$: *m/z* 240; and perylene-d12, $C_{20}D_{12}$: *m/z* 264). The capability of verifying mass spectral patterns as well as retention times make the GC/MS technique a very reliable method for PAH analysis.

The accuracy and precision of this single puff PAH analysis technique were first tested by a series of PAH standard solutions, then by replicate cigarette samples, and

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finally by comparison with cigarettes whose benzo[a]pyrene yield was known. A set of four PAH standard solutions was prepared with the toluene, hexane, isopropanol solvent mixture containing the internal standards. The concentration ranges were 0.2 to 2.0 μg/ml for naphthalene, 0.04 to 0.4 μg/ml for fluoranthene and 0.02 to 0.2 μg/ml for phenanthrene, pyrene and benzo[a]pyrene, equivalent to the concentration range occuring in the TPM of mainstream smoke of cigarettes collected with the single puff sampling technique. A triplicate of each standard solution was analyzed and the resulting curve of normalized response, i.e. the integrated PAH peak area divided by the corresponding integrated internal standard peak area, versus concentration was linear for all five PAHs with correlation coefficients of 0.999 or better. This good correlation between the normalized response versus concentration over the typical PAH levels in smoke warrants the use of normalized responses to represent the relative PAH levels in the single puff analysis.

We have chosen not to use calibration curves for quantification of PAH in this study. One of the reasons is the significant matrix difference between PAH standard solutions and the smoke samples. Our samples received no cleaning treatment as is usual in traditional PAH analysis. Instead, we compared the PAH yield of an unknown sample with a known control cigarette puff.

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Results and discussion

Cigarette analysis The typical yields per cigarette in mainstream smoke for the five PAHs analyzed in this study are reported to be 2-4 μg, 0.2-0.4 μg, 60-150 ng, 45-140 ng, and 9-40 ng, for naphthalene, phenanthrenes, fluoranthene, pyrene, and benzo[a]pyrene, respectively [11,13]. It should be noted that depending on cigarette design parameters such as tobacco type, paper characteristics, cigarette length, filter, filter ventilation, etc., the PAH yields will vary. We recently found that the total PAH yields of mainstream smoke of a cigarette are greatly affected by the types of flame used to light the cigarette, due to the contamination from the flames [8]. Since all the PAHs occur in minute amounts in mainstream smoke, it is technically challenging to analyze them in a single puff without extensive sample treatment. In this study, this difficulty was overcome by applying a minimum amount of solvent (50 μl) and choosing the mass spectrometry detector in selected ion monitoring (SIM) mode. Both sensitivity and selectivity are increased with SIM detection. Table 1 shows the relative PAH yields and reproducibility of the single puff PAH analysis technique applied to five IM16 cigarettes. The data were obtained from the fourth puff of each cigarette. The relative PAH yields were defined as the integrated PAH peak areas normalized to the response of the corresponding internal standard and divided by the amount of TPM produced in puff 4. It is interesting to note that the relative standard deviation (RSD) is the largest for the most abundant compound naphthalene. This could be due to the higher volatility of naphthalene, which influences its equilibrium between gas phase and particulate phase in smoke. Larger PAHs reside mostly in the particulate phase. Interference from untreated smoke matrix could be more severe in the lower molecular weight region, which may also contribute to the larger RSD observed for the smaller PAHs.

The good reproducibility of this method is illustrated by the low relative standard deviation (RSD) obtained for the PAH yield of a single puff (fourth puff) of IM16 cigarettes. In the benzo[a]pyrene measurements one extreme value was eliminated based on the Dixon Q Outlier Test [14,15]. Calibration curves for each PAH in the complex smoke matrix were not obtained. Instead, the method has been designed for comparison

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between different cigarettes on a puff per puff basis, and for comparison between different puffs or different lighting devices. The limit of detection for benzo[a]pyrene is estimated to be in the lower picogram range for this single puff analysis.

In order to verify that the single puff method for PAH analysis produces a linear response with the PAH concentrations in a complex matrix of untreated sample, single puffs of three types of reference cigarettes, including 1R4F, 99.KF.146, and 99.KF.182, were analyzed for benzo[a]pyrene (fourth puff of three cigarettes per type of cigarette). The relative benzo[a]pyrene levels, defined as the integrated benzo[a]pyrene peak area normalized to the response of the corresponding internal standard (perylene-d12, m/z 264) and divided by the amount of TPM produced in puff 4,

$$\text{Relative benzo[a]pyrene level} = \frac{\text{integrated benzo[a]pyrene peak area}}{\text{integrated perylene-d12 peak area} * \text{TPM (mg)}}$$

were measured at 0.015, 0.012, and 0.03 (mg^{-1}), for 1R4F, 99.KF.146, and 99.KF.182 cigarettes, respectively. The average B[a]P yield per cigarette for the same three types of cigarettes were also determined by a method where the collection of TPM of multiple cigarettes was followed by extensive sample treatment and analyzed by HPLC with fluorescence detector [16]. They were 0.96, 0.56, 1.94 (ng/mg TPM) for 1R4F, 99.KF.146 and 99.KF.182 cigarette, respectively. The single puff method for B[a]P analysis correlates well with the reference method based on multiple whole cigarettes [16], where

$$\text{B[a]P/TPM single puff } (mg^{-1}) = 0.013 \times \text{B[a]P/TPM (ng/mg)} + 0.004$$

has a correlation coefficient of 0.993. The result for benzo[a]pyrene is expected to be representative of other PAHs.

Lighting puff PAH analysis The relative yields of naphthalene, phenanthrene, fluoranthene, pyrene and benzo[a]pyrene in the lighting puffs (*i.e.* the first puff) of IM16 reference cigarettes were shown in Figure 1. Four different lighting devices were used, including an electric lighter, a butane torch lighter, a conventional butane lighter and paper book safety matches. To facilitate the comparison, the PAH yields from each lighting device were normalized against the electric lighter yields.

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Average values of three experiments were used for each compound. In most cases, the relative standard deviations were better than 20%. It should be pointed out that the lighting puff usually has the greatest uncertainty in measurement. This is due to the use of an external lighting device and the difficulties associated with establishing a consistent lighting manner. The cigarettes lit with match or butane lighter have higher concentrations of all PAHs tested in the lighting puff. The electric lighter and the butane torch lighter have similar PAH yields. It appears that this lighting device effect becomes more pronounced as the size of PAH increases. The difference is particularly striking in the case of benzo[a]pyrene where the increase exceeds an order of magnitude.

It is well known from hydrocarbon fuel flame studies that soot forms in the flame and that soot particles are covered with PAH molecules [17-23]. Siegman and Siegman [17] measured the distribution of selected molecules in a methane flame using time-of-flight (TOF) mass spectrometry. Smaller molecules such as acetylene and benzene were found to be spread all over the flame whereas larger molecules such as pyrene ($C_{16}H_{10}$) and perylene ($C_{20}H_{12}$) were concentrated toward the tip of the flame. The height profiles show that the position of maximum concentration rises with PAH size. PAH species constituting up to 12 benzene rings were well within the flame height.

It is not difficult to explain why different lighting devices have different PAH yields in the lighting puff. Matches and the butane lighter are representative of devices that produce yellow-tipped flames. The yellow portion of the flame is due to emission by incandescent carbon and, therefore, is characteristic of carbon cluster formation and indicative of incomplete combustion [24,25]. Polycyclic aromatic hydrocarbon formation is associated with this portion of the flame. With matches and the butane lighter, mixing occurs primarily by diffusion and, hence, the burning process is slow. In contrast, with the torch lighter the fuel gas is discharged at high velocity, the flow becomes more turbulent and mixing is accelerated. Combustion is more rapid and complete in comparison to the match or butane lighter. The yellow portion of the flame associated with polycyclic aromatic hydrocarbon formation is absent in the torch lighter. PAH formation in the flame is minimized. For example, the torch lighter flame produces at least 30 times less benzo[a]pyrene by itself, compared to the match or yellow flame

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butane lighter [26]. With the electric lighter, flame is completely absent. We have found that some soot particles from the match or the yellow flame butane lighter are carried by mainstream smoke through the cigarette and out the filter [8]. When a match or a butane lighter is used to light a cigarette, a large portion of the PAHs detected in the first puff is contributed by the flame, either directly from the flame or by interactions of the flame with tobacco or compounds produced in tobacco burning. Since the larger PAHs such as pyrene and benzo[a]pyrene are less abundant compared to the smaller ones in cigarette smoke, the contributions from the yellow flames in the cases of the match and butane lighter become more predominant in the lighting process and therefore the lighting device effect is more pronounced for larger PAHs.

Single puff benzo[a]pyrene of a reference cigarette Benzo[a]pyrene, occurring in 9-40 ng per cigarette, is the most extensively studied PAH in cigarette smoke [13,27]. The relative per puff benzo[a]pyrene levels for the IM16 cigarette were measured by the single puff analysis technique and shown in Figure 2. The relative benzo[a]pyrene level was defined in this study as the integrated benzo[a]pyrene peak area normalized to an internal standard (perylene-d12, m/z 264, 1μg/ml) and TPM (mg) from the puff. The addition of the internal standards in the sample helps to minimize the instrument variations and to increase the reproducibility of measurement. Four different lighters were used to light the cigarettes, including an electric lighter, butane torch lighter, conventional butane lighter and paper book safety matches. Eight puffs from the same cigarette were collected individually and analyzed for each lighter. The analysis was performed in random order among the eight puffs to minimize the uncertainties from instrument variations. Three cigarettes were analyzed for each lighting device, and the averages and standard deviations of the relative benzo[a]pyrene levels were shown in Figure 2. For torch lighter and electric lighter, no significant difference was observed among the puffs. As would be expected, the increase in benzo[a]pyrene with matches or the yellow flame butane lighter comes almost entirely from the lighting puff although there may be a slight carryover to the second puff. The levels observed in the first puff with matches or the butane lighter are approximately equal to the sum of the rest of the

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puffs. This is in good agreement with the factor of two difference observed for the whole cigarettes by the quantitative analysis method where an average of eight puffs was collected for one IM16 cigarette [8]. For later puffs the benzo[a]pyrene levels seem independent of lighting device within the sensitivity of this single puff analysis technique.

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Conclusion

We developed a single puff polycyclic aromatic hydrocarbon (PAH) analysis technique and applied it to study PAH formation and distribution in the cigarette smoking process. This single puff analysis method correlates well with whole cigarette analysis method. Information on PAH yield in each individual puff of a cigarette was obtained. Devices used to light cigarette were found to play a significant role in the PAH levels in the first puff as well as in the whole cigarette.

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References

1. R. F. Severson, M. E. Snook, O. T. Chortyk, and R. F. Arrendale, *Beitr. Tabakforsch.* 8: 273 (1976).
2. V. Norman, *Rec. Adv. Tob. Sci.* 3: 28 (1977).
3. O. T. Chortyk and W. S. Scholtzhauer, *Beitr. Tabakforsch.* 7: 165 (1973).
4. I. Schmeltz, J. Tosk, and D. Hoffmann, *Anal. Chem.* 48: 645 (1976).
5. R. R. Baker, *Thermochimica. Acta* 17: 29 (1976).
6. H. R. Burton and G. Childs, Jr., *Beitr. Tabakforsch.* 8: 174 (1975).
7. G. M. Badger, J. K. Donnelly, and T. M. Spotswood, *Austr. J. Chem.* 18: 1249 (1965).
8. S. Li, J. L. Banyasz, R. M. Olegario, C. B. Huang, E. A. Lambert and K. H. Shafer, *Combust. Flame* 128: 314 (2002).
9. S. Li, J. L. Banyasz, M. E. Parrish, J. Lyons-Hart, and K. H. Shafer, *J. Anal. Appl. Pyrolysis*, in press.
10. M. E. Parrish and C. N. Harward, *Appl. Spectrosc.* 54: 1665 (2000).
11. R. R. Baker, in *TOBACCO: Production, Chemistry, and Technology,* D.L. Davis and M.T. Nielsen, Eds., Blackwell Science, Inc., Malden, MA, 1999, pp. 398-439.
12. J. N. Diana and A. Vaught, *The Research Cigarette*, University of Kentucky Press, Lexington, KY, 1990.
13. D. Hoffmann and I. Hoffmann, *J. Toxicol. Environ. Health* 50: 307 (1997).
14. J. C. Miller and J. N. Miller, *Statistics for Analytical Chemistry*, 3rd Edition, Prentice Hall, 1993.
15. V. Barnett and T. Lewis, *Outliers in Statistical Data*, John Wiley & Sons, 1978.
16. CORESTA draft method, Determination of Benzo[a]pyrene in Total Particulate Matter of Tobacco Smoke, similar to method CAN/CGSB-176.2 No.1-96, March 1996 of the Canadian General Standards Board, National Standard of Canada.
17. H. Richter, W. J. Grieco, and J. B. Howard, *Combust. Flame* 119: 1 (1999).
18. K. Siegmann and H. C. Siegmann, *Europhysics News* 28: 50 (1997).
19. K. Nikolaou, P. Masclet, and G. Mouvier, *Sci. Total Envirn.* 32: 103 (1984).
20. L. R. Glass and C. E. Easterly, in *Polynuclear Aromatic Hydrocarbons: Measurements, Means, and Metabolism, 11th Int. Symp. on Polynuclear Aromatic*

PM3001141854

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Hydrocarbons (Cooke, M., Loening, K., and Merritt, J., Eds.), Battelle Press, Columbus, OH, 1991, p. 341.

21. N. Olten and S. Senkan, *Combust. Flame* 118: 500 (1999).
22. B. J. Finlayson-Pitts and J. N. Pitts, Jr., *Science* 276: 1045 (1997).
23. R. Hammerle, D. Schuetzle, and W. Adams, *Environ. Health Perspect.* 102: 25 (1994).
24. B. Lewis and G. von Elbe, *Combustion, Flames and Explosions of Gases*, Academic Press, Inc., Orlando, FL, 1987.
25. J. A. Barnard and J. N. Bradley, *Flame and Combustion*, Chapman and Hall, New York, NY, 1985.
26. R. J. Evans, National Renewable Energy Laboratory, Golden, CO, and M. R. Hajaligol, Philip Morris USA, Richmond, VA, personal communications.
27. D. Hoffmann and E. L. Wynder, *Cancer Res.* 27: 172 (1967).

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Figure Captions

Figure 1. Comparison of lighting device effect on five PAHs in IM16 cigarette smoke (Lighting puff, normalized to electric lighter yields).

Figure 2. Single puff relative benzo[a]pyrene yields of IM16 cigarettes using different lighting devices.

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Table 1. Single puff relative PAH yields per milligram of TPM of IM16 cigarettes (Puff #4)

	Naphthalene	Phenanthrene	Fluoranthene	Pyrene	Benzo[a]pyrene
Relative PAH yield[a]	1.092	0.312	0.098	0.085	0.014
% RSD (n=5)	12.2	5.8	5.3	3.0	3.2

[a] Relative PAH yields were the integrated PAH peak areas normalized to the response of the corresponding internal standard and divided by the amount of TPM produced in puff #4 of each replicate. For 5 replicates, average TPM = 1.55 mg/puff with RSD=7.9%.

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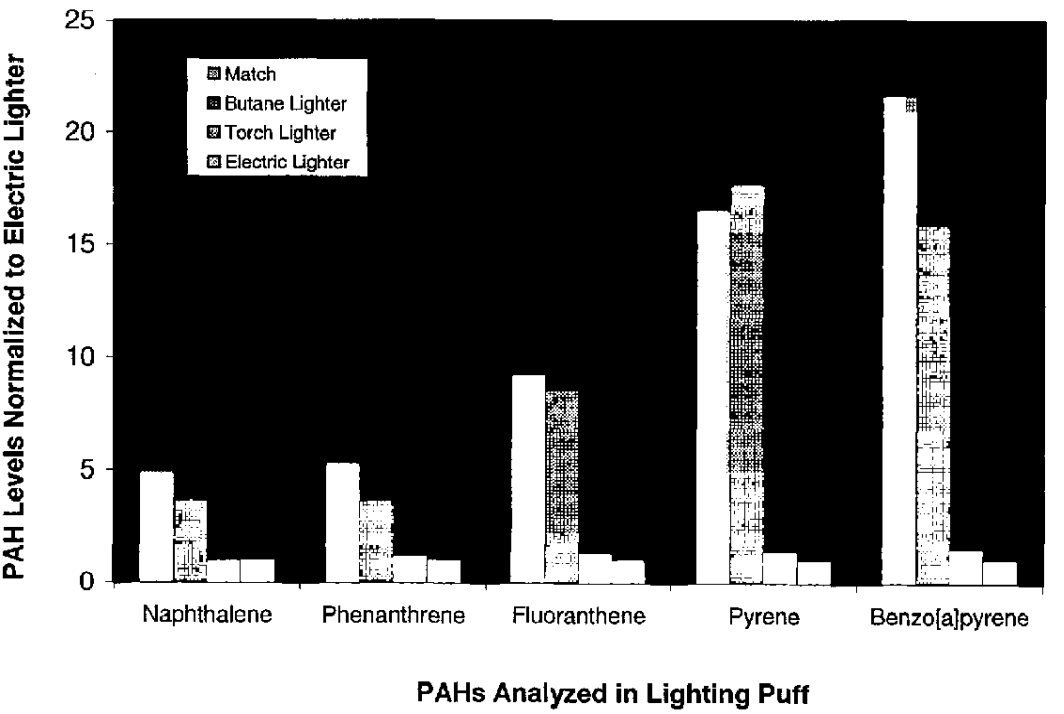

Figure 1. Comparison of lighting device effect on five PAHs in IM16 cigarette smoke (Lighting puff, normalized to electric lighter yields).

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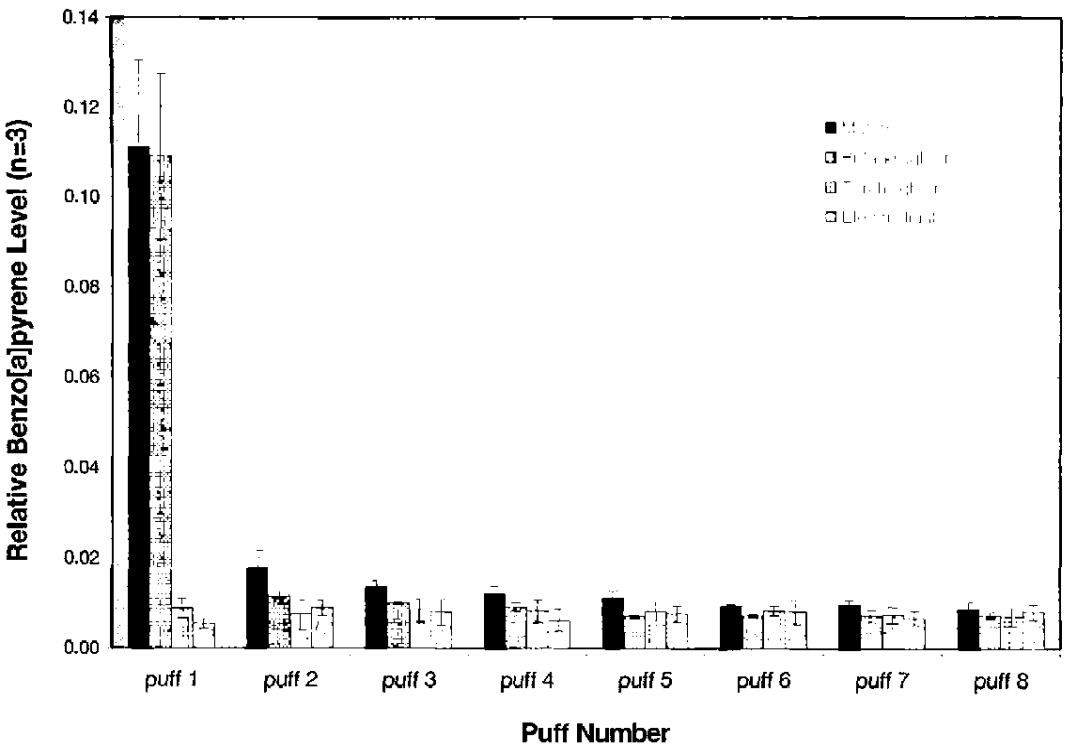

Figure 2. Single puff relative benzo[a]pyrene yields of IM16 cigarettes using different lighting devices.

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